Stressor-evoked brain and cardiovascular responses to stress.

NCT05413512

Date: 02/20/2020

Study Description

Title	Stressor-evoked brain and cardiovascular responses to acute psychological stress
Principal Investigator	Annie T. Ginty, Ph.D.
Study location(s)	Baylor University, Waco, Texas
Objectives	The main objective of Study 1 is to examine the relationship between cardiovascular, metabolic, and neural responses to acute psychological stress in healthy adults.
Main Inclusion/Exclusion Criteria	Participants may participate if they are between the ages of 18-30 and will be recruited from the surrounding area. Exclusion criteria will include: 1) any history of chronic medical or neurological disorder (e.g., traumatic brain injury, cardiovascular disease), 2) metal exposure, aneurysm clips, pacemaker, claustrophobia, cochlear implant or colorblindness, 3) current illness or infection, and 4) any condition that would prohibit them from engaging in a physical exercise testing session.

TABLE OF CONTENTS

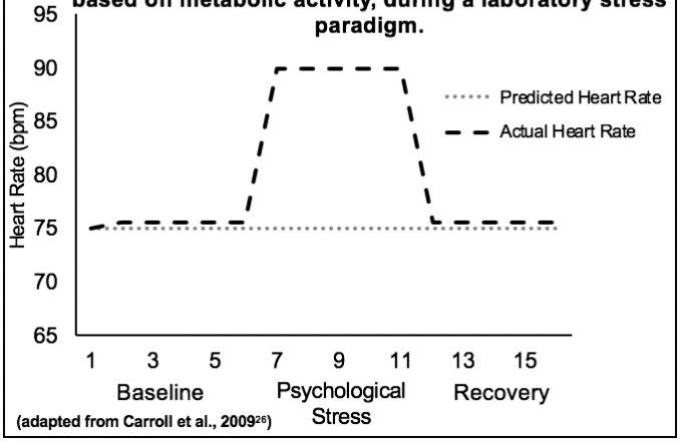
1.0 BACKGROUND AND RATIONALE

Significance: Cardiovascular disease (CVD) and cardiovascular outcomes are a leading burden to public health[1] and the risk levels of CVD from psychological stress are comparable to the level of risk conferred by smoking and physical inactivity[2]. A stressor-evoked cardiovascular reaction to psychological stress (i.e., rapid and autonomically mediated rises in blood pressure and heart rate) may be adaptive. However, some individuals have an exaggerated stress response, and we have shown that exaggerated cardiovascular reactions to stress predict CVD mortality[3]. Over the long-term, such exaggerated stressor-evoked cardiovascular reactions may initiate or exacerbate pathophysiological changes on the heart and vasculature[4]. Unfortunately, the current state of knowledge is strictly correlational. We lack an understanding of the neural basis for the generation of exaggerated cardiovascular reactions that are excessive to the immediate physiological needs of the individual. We expect to identify the initial neural correlates of how psychological stress-related processes instantiated in the brain relate to metabolically exaggerated cardiovascular stress responses, providing important groundwork for identifying how these processes may be intervened upon to reduce associated CVD risk.

Scientific Premise: The overall premise of the proposed work is based upon over three decades of research and meta-analyses suggesting that individual differences in cardiovascular responses to acute psychological stress are associated with future disease outcomes. Two key areas provide a basis for this project:

Autonomic responses to stress and future cardiovascular disease risk. There is longstanding and cumulative epidemiological evidence that individuals who exhibit a phenotype characterized by the expression of large-magnitude cardiovascular reactions are at elevated risk for clinical and preclinical endpoints of CVD[5-8]. My work and the work of others demonstrates that individual differences in these responses are relatively stable[9-13]. Moreover, cardiovascular responses during acute psychological stress, stress that does not require physical exertion, can be metabolically inappropriate—meaning that the cardiovascular system is working in excess of metabolic demand[14-24]. To determine if cardiovascular responses are metabolically appropriate, we measure oxygen consumption (marker for metabolic activity) and cardiovascular activity at rest and during a graded exercise test. The cardiovascular and metabolic responses to moderate exercise is a marker for metabolically appropriate heart rate changes[14]. Individual regressions of oxygen consumption on heart rate are calculated based on measurements during exercise. The computed regressions for each participant are then used with their oxygen consumption scores recorded during stress to "predict" what their heart rate should be during baseline and acute psychological stress. The difference between observed heart rate and predicted heart rate is called additional heart rate, which represents heart rate in excess of metabolic demand (i.e., the degree to which the heart is responding inappropriately or in excess during psychological stress). Figure 2 displays a representation of predicted heart rate versus actual heart rate during a standard psychological stress testing paradigm[14]. The lack of physical exertion during psychological stress does not perturb the metabolic system, which results in predicted heart rate being the same as rest. However, participants, on average, demonstrate an increase in heart rate in excess of the metabolic demand. A longstanding view is that the repeated and cumulative expression of such metabolically-disproportionate, stressor-evoked, cardiovascular reactions contribute to, or exacerbate, pathophysiological changes that are conducive to CVD and CVD events among vulnerable individuals[14-17]. Recently, it has been proposed that the brain generates anticipatory visceromotor commands to alter cardiovascular physiology to prepare individuals to cope with psychological stressors[25] and that these commands are "visceral predictions", insofar as to anticipate behavioral demands engendered by appraised stressors[26,27]. Individuals with metabolically disproportionate responses may be displaying visceral prediction errors[28]. The proposed work is both conceptual and empirical and provides a rationale for examining, in the context of CVD risk, the relationship between brain systems for visceral control and body metabolically exaggerated responses to stress.

Figure 2. Example of predicted vs. actual heart rate, based on metabolic activity, during a laboratory stress paradigm.



(adapted from Carroll et al., 2009[26])

Brain-imaging studies of stressor-evoked cardiovascular reactivity. Recent meta-analyses have identified core components of a broad network of forebrain systems involved stressor-evoked changes in cardiovascular activity[25,30-33]. These forebrain areas functionally interact with one another to modulate visceromotor and viscerosensory functions of cell groups within areas that govern and monitor autonomic and neuroendocrine outflow to the heart and blood vessels in coordination with behavioral actions and motivated dispositions to act[34-36]. Recent studies address previous limitations in stressor-evoked neuroimaging research by examining how patterns of responses, rather than voxel-by-voxel analyses, predict the likelihood of a certain magnitude of cardiovascular response[37,38]. Using multivariate and cross-validation methods allows for more rigorous statistical testing and more generalizable results that could identify a brain phenotype that reflects CVD risk[38,39]. Although it is the metabolically-disproportionate, stressor-evoked, cardiovascular reactions that are thought to most closely link with pathophysiological changes in vulnerable individuals, previous work in the field has only examined the neural correlates of stressor-evoked cardiovascular changes (i.e., cardiovascular reactivity)[9,25,37,40-42]. This study will be the first study to directly test the hypothesis that metabolically-disproportionate, stressor-evoked, cardiovascular reactions are associated with 'visceral prediction' errors[28].

2.0 STUDY OBJECTIVES

The main objectives of this study are to examine cardiovascular, metabolic, and neural responses to acute psychological stress. Additionally, the long-term goal of the study is to identify the multivariate pattern of stressor-evoked brain activity that reliably predicts individual differences in metabolically excessive responses to acute psychological stress.

3.0 SUBJECT SELECTION & RECRUITMENT

3.1 INCLUSION CRITERIA

One hundred and fourty participants (at least 50% female) between the ages of 18-30 years will be recruited from the surrounding area.

The age range was selected based on a recent meta-analysis by my colleagues and I, which demonstrated that participants ages 18-30 have different cardiovascular reactivity compared to those over 30[43]. The sample will be representative of the greater Waco demographics.

3.2 EXCLUSION CRITERIA

Exclusion criteria will include: 1) any history of chronic medical or neurological disorder (e.g., traumatic brain injury, cardiovascular disease), 2) metal exposure, pacemaker, claustrophobia, cochlear implant, or colorblindness 3) current illness or infection, and 4) any condition that would prohibit them from engaging in a physical exercise testing session.

3.3 RECRUITMENT

We plan to recruit participants to test over a four-year period. This should provide ample time to recruit participants. It also allows us time to alter our recruitment efforts if we are not meeting targets. We will evaluate recruitment every two months to make sure we are on track to hit our targeted number of participants. Based on previous work by our laboratory and others in the area, we will use recruitment strategies that are effective within our community. Specifically, we will advertise for participants at Baylor University using the participant subject pool, on flyers across campus, through ads at local shops and stores, and on local social media sites.

3.4 CONSENT

Written consent form: Participants will be emailed a consent form and asked to read it before coming into the laboratory (see participant consent form). The purpose of sending the consent form prior to the study is to ensure participants have a proper amount of time to consider their participation and consent. The email will also include information about what the participant should do to be prepared for the visit: no vigorous exercise or alcohol within 12 hours of participating in the study, no caffeine, food, or drink within 2 hours of the study (water is ok), and no current illness (e.g., strep throat). Participants will also be asked to wear comfortable, loose fitting clothes that they can exercise in (to aid in ease of putting on blood pressure cuff and electrodes and for exercising).

At the beginning of their first scheduled visit, participants will be presented with the consent form again and the experimenter will walk the participant through the consent form and the protocol of the study (i.e., explain what will happen during the study). The experimenter will also go over all risks associated with the study and ensure that the participant understands the risks. During this period, the participant will be encouraged to ask the experimenter any questions. The participant will then be asked to sign the consent form indicating that they understand the details of the study and consent to participating in the study before they can participate in the actual study.

The protocol will be the same for all participants (i.e., there will be no randomization). Participants have the right to withdrawal at any point during the study. They will be notified that they will receive their $50 at the completion of the neuroimaging portion of the laboratory.

4.0 RESEARCH DESIGN, METHODS, AND STUDY ACTIVITIES

Rationale: Previous research has identified areas of the brain associated with psychological stress and cardiovascular responses to stress; however, this research has not examined what is thought to be the most detrimental responses to stress, known as metabolically exaggerated[28]. Participants will have their metabolic and cardiovascular responses to stress measured, as well as undergo an fMRI scan to examine their stressor-evoked neural responses. The hypothesis here is that a multivariate pattern of stressor-evoked responses will be able to predict the magnitude to which cardiovascular responses to stress are excessive of metabolic demand and that this pattern will encompass areas implicated in stress processing and cardiovascular control. This aim will be vital in elucidating the neurophysiological pathways of metabolically excessive cardiovascular responses to stress by identifying key brain areas that are related to the peripheral responses.

Design: The protocol will be the same for all participants.

Duration: Visit 1 will take approximately 2 hours and Visit 2 will take approximately 2 hours. The total commitment will be 4 hours.

Visit 1 Protocol: Visit 1 is designed to determine the additional heart rate (i.e., degree to which each participant's response to stress is metabolically inappropriate). Participants will come to the laboratory to engage in a standard stress testing paradigm. Following instrumentation with cardiovascular equipment and body metabolic measuring equipment, participants will be given 20 minutes to adapt to the laboratory environment. This will be followed by a 10-minute formal resting baseline, and 18-minutes of acute psychological stress tasks (MSIT and Stroop task), see description below[9]. This will then be followed by four 4-minute bouts of cycling exercise incrementally increasing in demand[14] and will include with a maximal exercise test (described below). Participants will fill out questionnaires regarding demographics and health behaviors.

Visit 1 Measurements: Cardiovascular activity will be measured continuously[43] throughout the protocol using electrocardiogram and will be calculated for each minute of the protocol using Mindware Kubios processing software[34]. Body metabolic rate, indexed by oxygen consumption, will be measured on a breath-by-breath basis assessing ventilation and analyzing inspired and expired gas composition using an integrated system. Metabolic rate will then be averaged for each minute. Additional heart rate will be calculated by first computing individual regressions of oxygen consumption on heart rate using data from rest and each of the 4-minute bouts of the graded exercise test[14]. The computed regressions for each participant will then be used, with each individual's observed oxygen consumption recorded during stress, to "predict" what heart rate should be during baseline and during the MSIT (acute psychological stress). The difference between observed heart rate and predicted heart rate will be additional heart rate, which represents heart rate in excess of body metabolic demand.

Visit 2 Protocol: Visit 2 is designed to measure stressor-evoked neural activity, required to develop multivariate analyses to predict metabolically excessive responses (i.e., additional heart rate). The same 150 participants will come to the VISN 17 CoE to engage in a functional magnetic resonance imaging protocol. The protocol will consist of a structural scan, followed by a functional scan during which participants will engage in the MSIT and Stroop.

Visit 2 Measurements: Functional and structural images will be obtained on a Philips Ingenia 3.0 Tesla Omega MRI scanner equipped with a 32-channel head coil. Blood-oxygen level-dependent (BOLD) images will be acquired over the stressor

period with the following parameters: FOV = 205 x 205 mm^2, matrix size = 64 x 64 mm^2, TR = 2,000 ms, TE = 28 ms, and FA = 90°. Functional scans will be obtained at rest and during an acute psychological stress task (MSIT).

Acute psychological stress tests: The MSIT is composed of two conditions: a congruent, control, condition and an incongruent, stress-inducing condition[9,42,45]. Participants are asked to identify the number that is different from two other numbers in a visual display by pressing 1 of three buttons on the response glove. Each finger corresponds to a specific number (1 = index finger, 2 = middle finger, 3 = ring finger). For the control, congruent conditions the number appears in a location that is aligned with the respective finger position. For the incongruent (stress) condition the correct number appears in a position that is not aligned with the answer (see Figure 4). To increase stressfulness, the task includes elements of time pressure and social evaluation[9]. The test has been shown to be comparable to other standard psychological stress tests[9], has good test-retest reliability[32], and reliably perturb the physiological system[9,38,32]. I have recently demonstrated through pilot testing that participants can engage in the MSIT while having metabolic activity measured and that there is strong test-retest reliability for measuring metabolically excessive heart rate responses to the MSIT. The Stroop interference test is similar to the MSIT, but colors are used instead of numbers[38]. Both tasks have been used extensively in research[38].

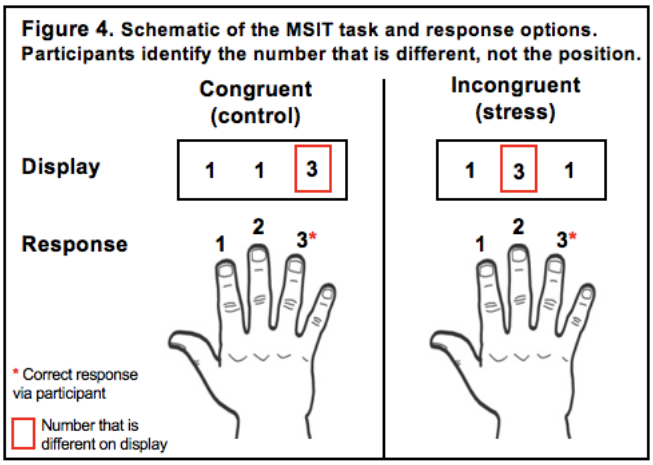


Figure 4. Schematic of the MSIT task and response options. Participants identify the number that is different, not the position.

Procedures and Activities for First Laboratory Visit:

- Experimenters will greet participants as they enter the lab and lead them to the lab waiting room.
- Once in the lab waiting room, participants will be given a copy of the consent form. The experimenter will walk the participant through the consent form and clearly explain all risks associated with the study. The participant will have the opportunity to ask any questions during and after the explanation (i.e., before signing the consent form). If the participant is still willing to participant in the study, he/she will sign the consent form.
- The experimenter will then set up the physiological equipment to measure blood pressure throughout the protocol. Participants will be asked to sit quietly for 10 minutes to adapt to the position of sitting. Blood pressure will be measured every 2 minutes on the non-dominant arm using a standard brachial artery cuff and a semi-automated sphygmomanometer for a total of 10 minutes. Before placing the blood pressure cuff on the arm of the participant, participants will be given the opportunity to ask any questions about the equipment. Participants will then be told the blood pressure cuff is similar to the one used at doctor's offices, with the exception that it will inflate with the push of a button (rather than someone pumping). Participants will be asked to sit quietly and the experimenter will inflate the blood pressure cuff once to ensure the participant is comfortable with the positioning and tightness of the cuff.
- The experimenter will then set up the physiological equipment to measure heart rate throughout the protocol. Electrocardiogram (ECG) will be recorded to determine heart rate. Three electrodes (circular stickers with a place in the center to attach wires) will be placed on the participants. Two electrodes will go just below the collar one and one electrode will go on the last rib on the left side. Participants will have the option to place the electrodes on themselves or have a researcher assist them. Three wires (one to each electrode) will then be attached and participants will be given a small box to clip on their pants (MindWare Mobile Impedance Cardiograph, Model 50-2303-00). The ECG data will be transmitted to a computer in which the trace of heart rate activity will be recorded. All data transmitted is de-identified.
- Participants will then be fitted with the face mask and hooked up to the Parvo Medics system to begin measures of their metabolic system. Oxygen consumption measured using an automated gas analysis metabolic system (Parvo Medics TrueMax 2400, Salt Lake City, UT) during a graded exercise test (standard Bruce protocol) on a treadmill (Spirit Medical MT200, Jonesboro, AR). Prior to each test, the gas analyzers will be calibrated with standard gases (O_2 and CO_2) of known concentrations.
- Once comfortable with the equipment set-up. Participants will begin the acute psychological stress and exercise paradigm. Below is a table outlining the paradigm.
- During the baseline periods, participants will be asked to sit quietly.

- The stress task will be the MSIT and Stroop (described above).
- The exercise portion will consist of a graded exercise test on a stationary bike (Monark, Sweden). Each exercise stage will be 4-minutes in duration and the participants will be requested to maintain a pedal rate of 50 revolutions per minute throughout the test. For the first 4 minutes, there will be no friction load on the wheel, but for each subsequent exercise stage, increasing friction loads will be applied to yield exercise power demands of 30, 60, and 90 W, respectively[14]. All participants will be requested to complete these initial four exercise stages. After the fourth stage, friction load will continue to increase in 30 W increments every 4 minutes until the participant reaches volitional exhaustion. Ratings of perceived exertion (RPE) (6-20 point Borg category RPE scale)[46] will be measured at the end of each 4 minute stage. Cardiovascular fitness will be determined from each participant's maximal oxygen consumption (VO_{2max}) measured using an automated gas analysis metabolic system (Parvo Medics TrueMax 2400, Salt Lake City, UT) and a Hans Rudolph face mask during the exercise test. Prior to each test, the gas analyzers will be calibrated with standard gases (O_2 and CO_2) of known concentrations. Expired gases will be monitored throughout the test. VO_{2max} will be determined by averaging the 2 highest consecutive 20 sec oxygen (O_2) consumption readings recorded, which will be subsequently compared to age- and gender-specific normative values (American College of Sports Medicine, 2018). The test will be deemed to be maximal providing it satisfies 3 or more of the following criteria: leveling of oxygen consumption, respiratory exchange ratio (RER) > 1.1, HR within 10 beats of the age predicted maximum and volitional exhaustion (RPE $\geq$ 17).
- After completing the exercise, participants will have the equipment removed and then have the opportunity to sit quietly until their heart rate goes back down to resting.
- Participants will then have anthropometric measurements taken:
    - Height and Weight: Total body weight (kg) and height (cm) will be determined on a standard dual beam balance scale and stadiometer (Detecto), respectively. Body weight will be recorded to the nearest 0.1 kg and height to the nearest 0.1 cm.
    - BMI: BMI will be determined from weight and height, where BMI equals weight (kg) / height (m^2). All BMI results will be recorded to the nearest 0.1 kg/m^2.
    - Waist circumference: Since waist circumference is positively correlated with abdominal fat content, this will be used as an adjunct marker of obesity for recruitment purposes. When standing at the participant's right side, a measuring tape will be extended parallel to the floor around the waist at the uppermost border of the right iliac crest. The measurement will be read to the nearest 0.1 cm at the end of the participant's normal expiration. A minimum of 2 measurements will be taken; however, if these vary by more than 2 mm a third measurement will be performed. Participants will also be given the option to place the tape measure around their own waist and hip for measurements if they would feel more comfortable. Every effort will be made to ensure male participants will have this measured by male RAs and female participants by female RAs. The participant is always given the option to take their own measurements with instructions from the RAs. There are always two RAs present during testing and the participant has the measurements taken in a private room (i.e., with two RAs and no other participants).
- Participants will also fill out questionnaires regarding demographics and health behaviors.

<u>Procedures for the Second Laboratory Visit:</u>

- Experimenters will greet participants as they enter the lab and lead them to the lab waiting room.
- Experimenters will walk the participants through the protocol of what will happen during the visit and give participants the opportunity to ask any questions they may not have asked during the first visit.
- Experimenters will then explain to participants the importance of removing any metal on their body and give them the opportunity to go into the changing room and remove any clothing with metal. Extra clothes without metal (basketball shorts and large t-shirts will be available if participants need them).
- Participants will then be walked into the scanning room and set-up for the scanning. The experimenter will ensure that the participant feels safe and comfortable in the scanning environment. If, for any reason, the participant appears to be becoming claustrophobic, they will be removed.
- Participants will be fitted with a blood pressure monitor and pulse oximeter to measure cardiovascular activity.
- Participants will engage in a 5 minute structural MRI scan, followed by functional scans during which they will engage in the MSIT (described above).
- After completion of the study, participants will be debriefed and have the opportunity to ask any questions.
- They will then be thanked for their time and provided with their payment.

Deception: The deception in the study has to do with the acute psychological stress task. Participants are told that they are being videotaped and that independent body language experts will review the videotape to analyze for signs of anxiety. However, the video camera is not actually recording during the task. Research suggests the addition of social evaluation in a laboratory stress paradigm makes the stress task more "life-like" and elicits a greater anxiety response[47]. It is important to emphasize that although participants think they are being video recorded, they are not actually being video recorded and no body language expert will review the tape. The PI has used this deception in multiple studies at Baylor University. Participants will not be made aware of this deception until after the second laboratory visit due to the same deception taking place during both visits.

Debriefing: At the end of the study visit participants will receive debriefing. During the debriefing period, participants will be told that the video camera was not actually taping them and will be informed that this was meant to help evoke "stress" in the stress condition. If a participant is upset at the close of the debriefing, they will be referred to the counseling center and an incident report will be submitted. The debriefing sheet is included as a separate document in this application. Participants will also be provided with the PI's information should they want to contact her regarding the deception.

5.0 RISKS & BENEFITS

Study 1 Risks

- Exercise Task:
  - Some participants may find it difficult to complete the exercise portion of the task. Individuals who have been advised not to exercise by a doctor are excluded from participating in the study. Participants will be told they can stop at any time if they are feeling ill from exercise. Additionally, participant's perceived exertion will be measured to ensure they are not at their maximum exertion for too long. In extreme cases, participants may experience cardiac problems associated with exercise. There is a defibrillator in the building where exercise testing will occur. Participants who meet eligibility criteria will be required to perform a sub-maximal exercise test. The participants will likely experience muscle soreness in their legs for between 24 to 48 h after exercise. This soreness is completely normal, and as these individuals will be inexperienced exercisers, they will receive ample warning regarding the risk.
  - Muscle strains or pulls resulting from the exercise testing sessions are also possible. However, prior to and during each exercise session, the participants will be familiarized with the exercise protocol and the correct technique required to perform the exercise safely on the treadmill. Therefore, we aim to minimize potential injury through proper instruction and protocol familiarization.
  - Trained, non-physician exercise physiologists certified in CPR will supervise participants undergoing testing and assessments. An automated electronic defibrillator (AED) is located in the Baylor Science Building and no less than two researchers will work with each participant during testing sessions. In the event of an emergency, one researcher will check for vital signs and begin intervention if required, while the other researcher contacts Baylor's campus police at extension 2222. All emergency procedures are posted above the phones in the event any other research investigator(s) is at hand to provide assistance.

- Stress Task:
  - The risks associated with the acute psychological stress task are minimal. The paradigm being used has been tested on thousands of people and no adverse side effects, other than feeling stressed during the task, have been experienced.

- functional Magnetic Resonance Imaging (fMRI):
  - There are some inherent risks associated with MRI in regard to metal implants. Entering the scanner with metal can cause risk to the machine and the participants.
  - We will ensure that all participants undergo a thorough screen to determine eligibility for the fMRI. Participants who have metal within them such as: aneurysm clips or pacemakers, will be excluded. There are known dangers from MRI to people with heart pacemakers, with the possibility that the pacemaker may malfunction, as well to people with metal implanted devices such as aneurysm clips that might be moved in a dangerous manner.
  - Participants will be asked questions about metal exposure throughout their lifetime and will be screened

with a metal detector to make sure they have not left any metal on their person.
    - There are no known effects of the magnetism or radio waves used in MRI.
    - Some people may become claustrophobic while in the machine. Should this occur, the examination will end and they will be quickly removed from the scanner. This is an occasional negative event that is usually mild in severity and quickly resolved upon leaving the scanner.

- Electrocardiography
    - Electrocardiography (ECG) carries minimal risk. However, some individuals may have skin irritation or an allergic reaction from the electrodes (sticky pads) being used. If you find the sticky pads are causing too much irritation, please let the researcher know and they can be removed.

- Loss of Confidentiality
    - A risk of taking part in this study is the possibility of a loss of confidentiality. Loss of confidentiality includes having your personal information shared with someone who is not on the study team and was not supposed to see or know about your information. The researcher plans to protect the participants' confidentiality through standard procedures noted in this form.

Incidental Findings:

- While the testing procedures the participants will undergo during this study are for research purposes only, there is the possibility the researchers may note a result potentially important to the participant's health. In this event, the participant in question will be contacted to explain the observation. If they do not have a private physician, we will refer them to an appropriate clinic for follow-up. Proceeding with any additional tests and/or treatments, if required, will be left to their discretion, and the participant or their insurance company will be responsible for any costs incurred.

Benefits:

- Possible benefits include participants learning a little bit more about themselves and how they cope with stress.
- Participants will be made aware of the proposed benefits of the study. All benefits will receive a copy of their personal results, which include: height, weight, BMI, and level of cardiovascular fitness. Participants will also have the opportunity to receive a printed picture of an image of their brain from the structural scan.
- Others may benefit in the future from the information that is learned in this study. Information from this study may be used to develop interventions to help people cope with stress.

6.0 STATISTICAL ANALYSIS

All participants will receive a unique ID for the study. IDs will be given starting with the number 9000. Questionnaire data will be collected through the Qualtrics survey system using tablets in the laboratory. Qualtrics will store the data on an encrypted server that will only be accessed by the Co-Investigator, primary RA, and students working on the study.

Analytic plan: Averages of the main study variables will be calculated for the main analyses of examining cardiovascular, metabolic, and neural activity at rest and during the acute psychological stress task. Reactivity will be calculated as: Stress activity – Baseline activity. fMRI data will be preprocessed and analyzed with statistical parametric mapping software (SPM12; http:www.fil.ion.ucl.ac.uk/spm) using established realignment, co-registration, smoothing, and motion correction analyses[38]. Stressor-evoked brain activation will be determined by comparing the BOLD signal activity during the incongruent (stress) versus congruent (control) condition[9,38]. For the analyses between stressor-evoked cardiovascular activity and stressor-evoked neural activity, machine learning techniques will be used to generate a multivariate pattern (3D regression weight map) that relates to heart rate and metabolic responses to stress. The study will then test if this generated multivariate pattern can reliability predict cardiovascular and metabolic responses to stress. Recent papers examining brain multivariate pattern prediction of cardiovascular responses to stress found the between subjects correlations to be relatively small, $r = \sim.20$. Based on these results, with alpha set at .05 and power = .80, it is estimated we will need a sample of at least $N = 140$.

7.0 DATA SECURITY & PRIVACY/CONFIDENTIALITY

All demographic data will be collected through Qualtrics. Qualtrics data is stored on an encrypted server.

- As per Qualtrics web page: "Qualtrics uses Transport Layer Security (TLS) encryption (also known as HTTPS) for all transmitted data. We also protect surveys with passwords and HTTP referrer checking. Our data is hosted by third party data centers that are SSAE-16 SOC II certified. All data at rest are encrypted, and data on deprecated hard drives are destroyed by U.S. DOD methods and delivered to a third-party data destruction service."
- All blood pressure data from the study will be written on a sheet of paper. The paper will only be labelled with the participant's unique ID number. Data from the paper will be entered into an encrypted SPSS file (again only under the unique ID number). The pieces of paper will be stored in a locked filing cabinet in the laboratory until the study is complete. This is in case someone needs to go back and double check that the written value matches the computer entered value while analyzing the data.
- All heart rate data will be stored under the unique ID number in encrypted folders. Raw heart rate data will be processed under these unique ID numbers and then aggregate (minute by minute) data will be entered into the same SPSS file as the blood pressure.
- All data are anonymized using the unique ID. A key linking the ID to the participant's name will be stored on an encrypted file on an encrypted hard drive. The rationale for this is much of the research the Baylor Behavioral Medicine Laboratory is interested in involves health and future health outcomes. In the future, it could be beneficial to contact participants for other studies measuring health and to link the data on their stress responses to this study to their future health. Participants will give consent for being contacted for future studies. The links between ID and name will not leave the Baylor Behavioral Medicine Laboratory and will only be accessed by those named on this ethics form.
- In the interest of open science, we desire to open our data to other researchers who wish include the data in meta-analysis or who wish to statistically replicate our analyses. No identifies will be collected.
- Data will be retained indefinitely, in the case that future meta-analytic researchers require additional information that is unreported in a future publication.

8.0 DATA & SAFETY MONITORING

Adverse Events: Trained, non-physician exercise physiologists certified in CPR will supervise participants undergoing assessments. An automated defibrillator (AED) is located in the Baylor Science Building and no less than two researchers will work with each participant during testing sessions. In the event of an emergency, one researcher will check for vital signs and begin intervention, if required, while the other researcher contacts Baylor's campus police extension 2222. All emergency procedures are posted above the phones in the event any other research investigator(s) is/are at hand to provide assistance.

A separate data safety monitoring plan has been created for each study. The data safety monitoring plan includes plans for protection against study risks, adverse reporting, and a safety office. Please see attached documents for data safety monitoring plan.

9.0 STUDY LOCATION

Visit 1 will take place in the Behavioral Medicine Laboratory located in the Baylor Sciences Building at Baylor University in Waco, Texas. Visit 2 will take place at the VISN 17 COE in Waco, Texas. This is not a multi-site study.

10.0 DISSEMINTATION PLAN

The principal investigator will ensure that the study is registered at ClinicalTrials.gov and that the results information from this study will be submitted to ClinicalTrials.gov for public posting.

11.0 REFERENCES

1. Mozaffarian, D., Benjamin, E.J., Go, A.S., Arnett, D.K., Blaha, M.J. […] Turner, M.B. (2016). Heart disease and stroke statistics- 2016 update: A report from the American Heart Association. *Circulation, 133,* e38-360. PMID: 26673558

2. Rozanski, A. (2014). Behavioral Cardiology: Current advances and future directions. *Journal of the American College of Cardiology, 64,* 100-110. PMID: 24998134.

3. Carroll, D., Ginty, A.T., Der, G., Hunt, K., Benzeval, M., & Phillips, A.C. (2012). Increased blood pressure reactions to acute mental stress are associated with 16-year cardiovascular disease mortality. *Psychophysiology, 49,* 1444-1448. PMID: 22958235

4. Chida, Y. & Steptoe, A. (2010). Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: a meta-analysis of prospective evidence. *Hypertension, 55,* 1026-1032. PMID: 20194301

5. Gerin, W., Pickering, T.G., Glynn, L., Christenfeld, N., Schwartz, A., Carroll, D., & Davidson, K. (2000). An historical context for behavioral models of hypertension. Journal of Psychosomatic Research, 48, 369-377.

6. Taylor, T.R., Kamarck, T.W., Dianzumba, S. (2003). Cardiovascular reactivity and left ventricular mass: an integrative review. *Annals of Behavioral Medicine, 26,* 182-193. PMID: 14644694

7. Treiber, F.A., Kamarck, T.W., Schneiderman, N., Sheffield, D., Kapuku, G., & Taylor, T. (2003). Cardiovascular reactivity and development of preclinical and clinical disease states. *Psychosomatic Medicine, 65,* 46-62. PMID: 12554815

8. Schwartz, A.R., Gerin, W., Davidson, K.W., Pickering, T.G., Brosschot, J.F., Thayer, J.F., & Christenfeld, N., Linden, W. (2003). Toward a casual model of cardiovascular responses to stress and the development of cardiovascular disease. *Psychosomatic Medicine, 65,* 22-35. PMID: 12444813

9. Ginty, A.T., Gianaros, P.J., Derbyshire, S.W., Phillips, A.C., & Carroll, D. (2013). Blunted cardiac stress reactivity relates to neural hypoactivation. Psychophysiology, 50, 219-229. PMID: 23351027

10. Hassellund, S.S., Flaa, A., Sandvik, L., Kjeldsen, S.E., & Rostrup, M. (2010). Long-term stability of cardiovascular and catecholamine responses to stress tests: an 18-year follow-up study. *Hypertension, 55,* 131-136. PMID: 199948985

11. Dragomir, A.I., Gentile, C., Nolan, R.P., & D'Antono, B. (2014). Three-year stability of cardiovascular and autonomic nervous system responses to psychological stress. *Psychophysiology, 51,* 921-931. PMID: 24853995

12. Cohen, S., Hamrick, N., Rodriguez, M.S., Feldman, P.J., Rabin, B.S., & Manuck, S.B. (2000). The stability of and intercorrelations among cardiovascular, immune, endocrine, and psychological reactivity. *Annals of Behavioral Medicine, 22,* 171-179. PMID: 11211850

13. Kamarck, T.W., Jennings, J.R., Pogue-Geile, M., & Manuck, S.B. (1994). A multidimensional measurement model for cardiovascular reactivity: stability and cross-validation in two adult samples. *Health Psychology, 13,* 471-478. PMID: 7889901

14. Carroll, D., Phillips, A.C., & Balanos, G.M. (2009). Metabolically exaggerated cardiac reactions to acute psychological stress revisited. *Psychophysiology, 46,* 270-275. PMID: 19207196

15. Balanos, G.M., Phillips, A.C., Frenneauz, M.P., McIntrye, D., Lykidis, C., Griffin, H.S., & Carroll, D. (2010). Metabolically exaggerated cardiac reactions to acute psychological stress: the effects of resting blood pressure status and possible underlying mechanisms. *Biological Psychology, 85,* 104-111. PMID: 20541585

16. Obrist, P.A. (1981). Cardiovascular Psychophysiology: A Perspective. Plenum Press, New York, NY.

17. Sherwood, A., Allen, M.T., Obrist, P.A., & Langer, A.W. (1986). Evaluation of beta-adrenergic influences on cardiovascular and metabolic adjustments to physical and psychological stress. *Psychophysiology*, 23, 89-104. PMID: 3003780

18. Turner, J.R. & Carroll, D. (1985). Heart rate and oxygen consumption during mental arithmetic, a video game, and graded exercise: further evidence of metabolically-exaggerated cardiac adjustments. *Psychophysiology, 22,* 261-267. PMID: 4011795

19. Lambiase, M.J., Dorn, J., Chernega, N.J., McCarthy, T.F., & Roemmich, J.N. (2012). Excessive heart rate and systolic blood pressure during psychological stress in relation to metabolic demand in adolescents. *Biological Psychology, 91,* 142-147. PMID: 22634388

20. Lambiase, M.J., Dorn, J., & Roemmich, J.N. (2012). Metabolic and cardiovascular adjustments during psychological stress and carotid artery intima-media thickness in youth. *Physiology & Behavior, 105,* 1140-1147. PMID: 22210396

21. Carroll, D., Harris, M.G., & Cross, G. (1991). Haemodynamic adjustments to mental stress in normotensives and subjects with mildly elevated blood pressure. *Psychophysiology, 28,* 438-446. PMID: 1745723

22. Delistraty, D.A., Greene, W.A., Carlberg, K.A., & Raver, K.K. (1991). Use of graded exercise to evaluate physiological hyperreactivity to mental stress. M*edicine and Science in Sport and Exercise, 23,* 476-481. PMID: 2056906

23. Langer, A.W., McCubbin, J.A., Stoney, C.M., Hutcheson, J.S., Charlton, J.D., & Obrist, P.A. (1985). Cardiopulmonary adjustments during exercise and an aversive reaction time task: effects of beta-adrenoceptor blockade. *Psychophysiology, 22,* 59-68. PMID: 3975320

24. Gianaros, P.J. & Jennings, J.R. (2018). Host in the machine: A neurobiological perspective on psychological stress and cardiovascular disease. *American Psychologist 73,* 1031-1044. PMID: 30394781 PMCID: PMC6220680

25. Gianaros, P.J. & Wager, T.D. (2015). Brain-body pathways linking psychological stress and physical health. *Current Directions in Psychological Science, 24,* 313-321. PMID: 26279608 PMCID: PMC4535428

26. Clark, A. (2013). Whatever next? Predictive brains, situated agents, and the future of cognitive science. The Behavioral and Brain Sciences, 36, 181-204. PMID: 23663408

27. Chanes, L. & Barrett, L.F. (2016). Redefining the role of limbic areas in cortical processing. *Trends in Cognitive Science, 20*, 96-106. PMID: 26704857 PMCID: PMC4780414

28. Ginty, A.T., Kraynak, T.E., Fisher, J.P., & Gianaros, P.J. (2017). Cardiovascular and autonomic reactivity to psychological stress: Neurophysiological substrates and links to cardiovascular disease. Autonomic Neuroscience, 207, 2-9. PMID: 28391987 PMCID: PMC5600671

29. Beissner, F., Schumann, A., Brunn, F., Eisentrager, D., & Bar, K. (2014). Advances in functional magnetic resonance imaging of the human brainstem. *Neuroimage, 86,* 91-98. PMID: 23933038

30. Muscatell, K.A. & Eisenberger, N.I. (2012). A social neuroscience perspective on stress and health. *Social and Personality Psychology Compass, 6,* 890-904. PMID: 23227112 PMCID: PMC3513933

31. Myers, B. (2017). Corticolimbic regulation of cardiovascular responses to stress. Physiology & Behavior, 172, 49-59. PMID: 27793557 PMCID: PMC5618801

32. Shoemaker, J.K. & Goswami, R. (2015). Forebrain neurocircuitry associated with human reflex and cardiovascular control. *Frontiers in Physiology, 6,* 240. PMID: 26388780 PMCID: PMC4555962

33. Thayer, J.F., Ahs, F., Fredikson, M., Sollers, J.J., & Wager, T.D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neuroscience and Biobehavioral Reviews, 36,* 747-756. PMID: 22178086

34. Bandler, R., Keay, K.A., Floyd, N., & Price, J. (2000). Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. *Brain Research Bulletin, 53,* 95-104. PMID: 11033213

35. Saper, C.B. (2002). The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annual Review of Neuroscience, 25,* 433-469. PMID: 12052916

36. Ulrich-Lai, Y.M. & Herman, J.P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews. Neuroscience, 10,* 397-409. PMID: 19469025 PMCID: PMC4240627

37. Eisenbarth, H., Chang, L.J., & Wager, T.D. (2016). Multivariate brain prediction of heart rate and skin conductance responses to social threat. J*ournal of Neuroscience, 36,* 11987-11998. PMID: 27881783 PMCID: PMC5125248

38. Gianaros, P.J., Sheu, L.K., Uyar, F., Koushik, J., Jennings, J.R., Wager, T.D., Singha, A., & Verstynen, T.D. (2017). A brain phenotype for stressor-evoked blood pressure reactivity. *Journal of the American Heart Association, 6,* e006053. PMID: 28835356 PMCID: PMC5634271

39. Woo, C.W., Chang, L.J., Lindquist, M.A., & Wager, T.D. (2017). Building better biomarkers: brain models in translational neuroimaging. *Nature Neuroscience, 30,* 365-377. PMID: 28230847

40. Wager, T.D., Waugh, C.E., Lindquist, M., Noll, D.C., Fredrickson, B.L., & Taylor, S.F. (2009). Brain mediators of cardiovascular responses to social threat: part I: Reciprocal dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity. *Neuroimage, 47,* 821-835. PMID: 19465137 PMCID: PMC3275821

41. Gianaros, P.J. & Sheu, L.K. (2009). A review of neuroimaging studies of stressor-evoked blood pressure reactivity: emerging evidence for a brain-body pathway to coronary heart disease risk. *Neuroimage, 47,* 922-936. PMID: 19410652 PMCID: PMC2743251

42. Sheu, L.K., Jennings, J.R., & Gianaros, P.J. (2012). Test-retest reliability of an fMRI paradigm for studies of cardiovascular reactivity. *Psychophysiology, 49,* 873-884. PMID: 22594784 PMCID: PMC3376344

43. Brindle, R.C., Ginty, A.T., Phillips, A.C., & Carroll, D. (2014). A tale of two mechanisms: a meta-analytic approach toward understanding the autonomic basis of cardiovascular reactivity to acute psychological stress. *Psychophysiology, 51,* 964-976. PMID: 24924500

44. Brindle, R.C., Ginty, A.T., Phillips, A.C., Fisher, J.P., McIntrye, D., & Carroll, D. (2016). Heart rate complexity: A novel approach to assessing cardiac stress reactivity. *Psychophysiology, 53,* 465-472. PMID: 26585809

45. Bush, G. & Shin, L.M. (2006). The Multi-Source Interference Task: an fMRI task that reliably activates the cingulo-frontal-parietal cognitive/attention network. *Nature Protocols,1,* 208-313. PMID: 17406250

46. Borg, G. (1998). Borg's perceived exertion and pain scales. *Human Kinetics.*

47. Veldhuijzen van Zanten, J.J.C.S., Ring, C., Burns, E., Edwards, K.M., Drayson, M. & Carroll, D. (2004). Mental stress-induced hemoconcentration: Sex differences and mechanisms. *Psychophysiology, 41,* 541-551.